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Electrophysiological properties of Arabidopsis thaliana guard cells

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Summary

In the outer cell layer of leaves stomates are located which form microscopic small pores in the leaf surface. These pores enable the exchange of gasses between plants and ambient air. In the light, carbon dioxide from the surrounding atmosphere enters the leaf where it is converted into sugars and other compounds. This process is called photosynthesis. During this process plants produce oxygen which is released through the same stomates. As a consequence of these open pores, water evaporates, which is often unfavourable for the plant. The degree of loss of water is regulated, within certain limits, by opening or closure of the stomates. So called stomatal movements cause the pore to widen or narrow. During drought, loss of water is prevented when stomates close, while opening of stomates occurs when there is a sufficient supply of water.

The hormone Abscisic Acid, or ABA, plays a key role in the response to drought. Plant tissues that sense a shortage of water release the hormone in response. The hormone is transported to the leaves by the transpiration stream, which is the flow of water from the roots through the stalk to the leaves. Within the leaves abscisic acid prevents opening of the stomates, or causes closure when stomates are already opened.

The goal of my research was to expand on our knowledge of the mechanisms by which ABA affects stomatal movements. For this, I studied the so called transport-processes underlying stomatal movements and the effect of abscisic acid hereupon, using electrophysiological techniques.

Plants of the species Tialcress or *Arabidopsis thaliana* were used for the research. Mutants of this species had been isolated with an altered sensitivity to abscisic acid as compared to the wild type (non-mutated plants). These mutants were named *abi*-mutants (abscisic acid insensitive). A lowered resistance of these plants to drought had already been reported for two of the *abi*-mutants, which were coded *abi1* and *abi2*.

The first goal was to establish whether the reduced drought resistance was due to insensitivity of the mutant stomates to abscisic acid. For this, the outer cell layer, in which the stomates are located, was carefully peeled from a leaf. In such a peel or strip the stomates remain intact. Opening of stomates in the peeled tissue could be induced by white light in both: wild type and mutants. Abscisic acid inhibited the opening of wild type stomates, but no inhibition by abscisic acid occurred in mutant stomates (Chapter 2). Stomates of *abi1* and *abi2*-mutants apparently are insensitive to the hormone, which explains the reduced drought resistance of these mutants.

Two stomatal guard cells that surround the stomatal pore determine its aperture. Guard cells are cylindrically shaped and bend when the cells swell, like

a stuffed sausage. Plant cells are enclosed by a cell wall. The bending of the cells is due to a lower elasticity of the cell wall facing the pore, as compared to the rest the of wall. The content of the cell applies pressure, or so called turgor, on this wall. When the turgor of guard cells increases the cells swell and thereby bend, which results in a wider stomatal pore. Changes in the turgor of guard cells are largely due to the uptake of potassium. Potassium is taken up as positively charged particles (K^+-ions). Uptake of K^+-ions is associated with an increase of turgor and it will therefore cause stomates to open.

The uptake of K^+ is driven by the efflux of other positively charged particles from the guard cells: protons. Guard cells pump protons across the outer membrane (plasma membrane) into the cell wall. A specialised protein, named proton pump, which drives the flux of the positively charged protons, is localised in the plasma membrane. As a result an electrical potential difference is created. This potential difference drives the uptake of K^+-ions by guard cells from their cell wall and surrounding solution into the cells. Activity of the proton pump increases the electrical potential difference and causes a higher rate of K^+-ions uptake.

The activity of the proton pump can be determined by measuring the acidity of the guard cell wall (Chapter 3). Changes in acidity reflect changes in the concentrations of protons. Light stimulates the activity of the proton pump, just as it stimulates the opening of stomates. A pulse of blue light acidifies the cell wall, which is the result of an increased activity of the proton pump. Abscisic acid inhibited the blue light induced acidification by wild type guard cells. However, the hormone had no effect on the acidification rate by *abi1* guard cells and it even stimulated the acidification by *abi2*. The proton pump of wild type guard cells is thus inhibited by abscisic acid, but the proton pump of *abi1* and *abi2* stomates is not.

The mutations of *abi1* and *abi2* have occurred in two different protein phosphatases. Protein phosphatases are often involved in regulatory processes in plant cells. Apparently the altered protein phosphatases are involved in the transduction of the abscisic acid signal, that finally leads to inhibition of the proton pump in the wild type.

As explained above, the electrical potential difference across the plasma membrane (the membrane potential) can drive the uptake of K^+-ions. The membrane potential can be measured by impalement of the cell with microelectrodes. Using double barrelled microelectrodes not only the membrane potential of a cell can be determined, but also its conductivity. The magnitude and direction of the K^+-flux across the plasma membrane can be determined, provided the membrane potential and the conductivity of the plasma membrane for K^+ are known.

Guard cells appeared to exist in two different states characterized by the

membrane potential that was measured with the impaled microelectrodes (Chapter 4 and 5). The majority of the cells had only a small potential difference across the membrane. These cells were labelled: cells in the depolarized state. Other guard cells had a much larger potential difference across the plasma membrane and were labelled: cells in the hyperpolarized state. Both states are important for the K^+-flux across the plasma membrane. Depolarized cells will have an efflux of K^+-ions, while hyperpolarized cells will take up K^+. Stomates with depolarized guard cells will therefore close, while stomates with hyperpolarized cells will open. Guard cells sometimes spontaneously made a transition from one to the other state. A few cells displayed repetitive transitions between the two states. These transitions are very likely essential for changes in the stomatal aperture in intact plants, "in vivo".

Specialised proteins, called ion-channels, conduct K^+-ions across the plasma membrane, while the membrane itself or matrix is impermeable for ions. Ion channels can be selective regarding the type of ions they conduct. The conductivity of many ion channels depends on the membrane potential. At a certain membrane potential these channels activate, thereby increasing their conductivity. In stomatal guard cells of Tialcrest three different types of K^+-conducting ion channels were identified (Chapter 4). Two of these channels activated at membrane potentials at which the K^+-flux was directed from the cell to the cell wall (outward). The other K^+-channel conducted a flux in the opposite direction (inward). This inward K^+-channel was only observed in cells in the hyperpolarized state.

As explained, the direction of the K^+-flux across the plasma membrane and the conductivity of the plasma membrane are both determined by the membrane potential. In its turn, the membrane potential depends on the activity of ion channels in the plasma membrane. The effect of changes in K^+-channel activity on the membrane potential was studied (Chapter 5). Changes in the acidity of the cell wall appeared to affect the activity of two K^+-channels. The membrane potential of depolarized cells depended on the activity of the slow outward K^+-channel, while the activity of the inward K^+-channel affected the membrane potential of hyperpolarized cells. Changes in the activity of K^+-channels, however, had little effect on the state in which the guard cells existed. The direction of the K^+-flux was apparently not determined by the activity of K^+-channels.

The activity of the proton pump is of importance for the direction of the K^+-flux across the plasma membrane. Activity of the proton pump is essential to hyperpolarize cells, since it is the only transporter in guard cells that can cause the large potential difference. Abscisic acid inhibits the proton pump and guard cells will therefore be less often in the hyperpolarized state. However, no inhibition of the proton pump occurred in guard cells of the two *abi*-mutants. In

the presence of abscisic acid these cells will thus remain as often in the hyperpolarized state , or even more often (*abi2*), as in its absence. As a consequence, the opening of stomates in these mutants is not inhibited by abscisic acid. Stomates will open regardless of the supply of water and mutant plants thereby wilt rapidly during drought.